

Accelerating your process optimization: sampling from reactions in-progress means better decisions in less time

Introduction and background

Drug development and fine chemical researchers are always searching for the best reaction conditions – those that give the greatest yield, the highest purity or the most reaction control, using the most cost effective reagents and conditions. Getting detailed knowledge of how a reaction performs under a variety of conditions is ideal, but the level of detail needed to make optimal process decisions is usually unobtainable due to time and resource constraints. As a result, your process is limited by inadequate information. Times have changed and technology exists that can give you a better understanding of a reaction profile and enable critical scale-up and control strategies.

The Optimization Sampling Reactor (OSR) from Unchained Labs is a proven automation tool that lets researchers study reaction kinetics, track conversion and impurity formation and determine the reaction end-point over short and long time scales, all without running extra reactions or having to use large amounts of material. You can get the right data and enough of it, which allows you to optimize processes faster while increasing scale up success. In this application note, we demonstrate how the OSR technology was used in our search for the best chemistry for OSR validation.

The Optimization Sampling Reactor

The OSR module (Figure 1) is comprised of eight 40 mL overhead stirred reactors, each with a

5–25 mL working volume range. Each reaction vessel can be independently heated to 150 °C, cooled to -20 °C and pressurized from ambient to 400 psi above ambient to tightly controlled limits. The automation allows samples to be taken from all eight reactors without halting the process – even under high temperatures and pressures, and with minimal loss of solvent or change of mass balance.

Scientists can easily build kinetic reaction profiles, detect any potential impurities and optimize these reactions with minimal material and time requirements. All results are funneled to a central database through the Lab Execution and Analysis (LEA) software suite. LEA, Unchained Labs' enterprise data solution, is an end-to-end solution that captures all relevant data from an experiment and stores them in a central database under one experimental ID. Data that spans from initial reac-

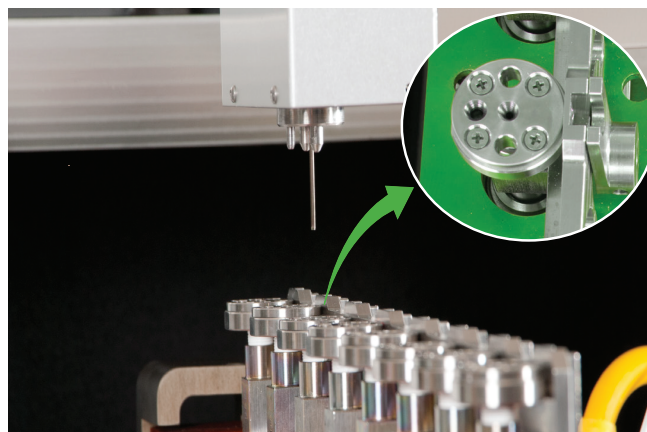


Figure 1: The Optimization Sampling Reactor (OSR) and controlled access sampling port.

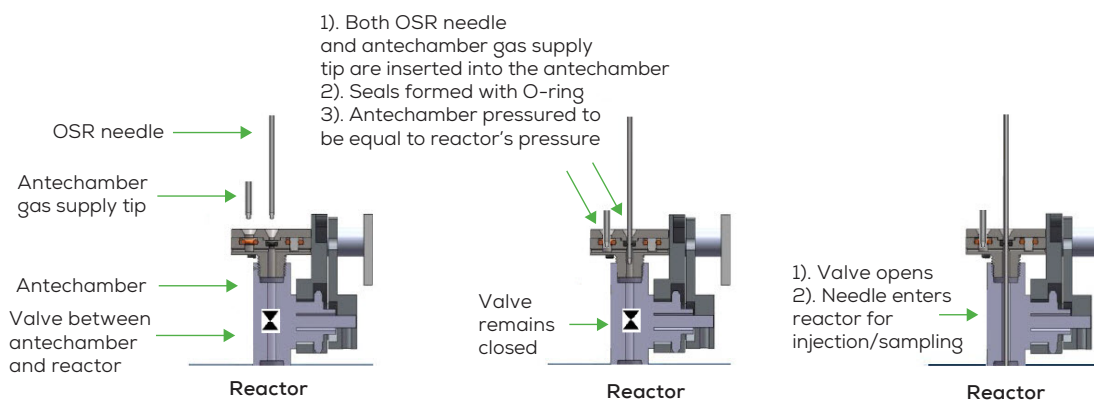


Figure 2: Controlled atmosphere injection and sampling under pressure.

tion design to OSR processing conditions and even analytical results (such as HPLC from third party equipment) can be viewed and analyzed from anywhere for more efficient reporting and seamless knowledge sharing.

Accurate sampling and dispensing at elevated pressures

Pressurized chemistry usually provides a technical challenge for researchers who want to remove time series samples from their reactions to study kinetics and impurity profiles. The compromise is often to replicate the reaction multiple times and quench each reaction at a desired time point. This approach is not only time-consuming, but can also result in more variability due to inconsistency in setting up replicates. Accurate, automated *in-situ* sampling and dispensing of new reagents, even at high temperatures and pressures, is a much better approach. Each condition is run only once and many sampling and dispensing points can take place during the run as desired, using the OSR's controlled atmosphere sampling port technology (Figure 2).

In order for the OSR needle to inject or sample from each reactor under pressure, it has to first enter an antechamber for pressure equilibration. First, the needle and antechamber gas supply tip are inserted into the antechamber under ambient pressure. There are O-rings for both to ensure pressure seals when the needle and gas supply tip enter. The antechamber then is pressurized via the gas supply tip to

reach the reactor's pressure. Next, a normally closed valve is actuated after pressure equilibrium to let the needle move into the reactor. The stirring paddle for the OSR (Figure 3) has a hollow shaft, allowing the needle to travel through without interfering with the stirring mechanism. Dispensing occurs at the top of the reactor while sampling takes place near the very bottom. This design feature also allows sampling to take place with continuous stirring (slurry sampling) which is ideal for obtaining representative samples from heterogeneous reactions. Under a different scenario, the stirring can also stop completely before sampling, allowing heterogeneous catalysts to settle so no catalyst is taken out of the reaction. In addition to time point sampling, the OSR can also monitor gas uptake in real-time and provide scientists with an additional method to track reaction progress for pressurized reactions.



Figure 3: OSR stirring paddle.

Hydrogenation of *trans*-Cinnamic acid

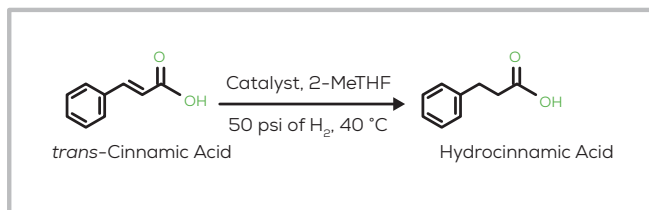


Figure 4: Hydrogenation of *trans*-Cinnamic acid.

For every OSR built, a robust and easy-to-perform chemistry is needed to evaluate its performance both in-house and at a customer site when delivered. Catalyzed hydrogenation of *trans*-Cinnamic acid is chosen due to its simplicity (Figure 4). There are only four components needed for this chemistry: *trans*-Cinnamic acid as the starting material, H₂ gas, catalyst and

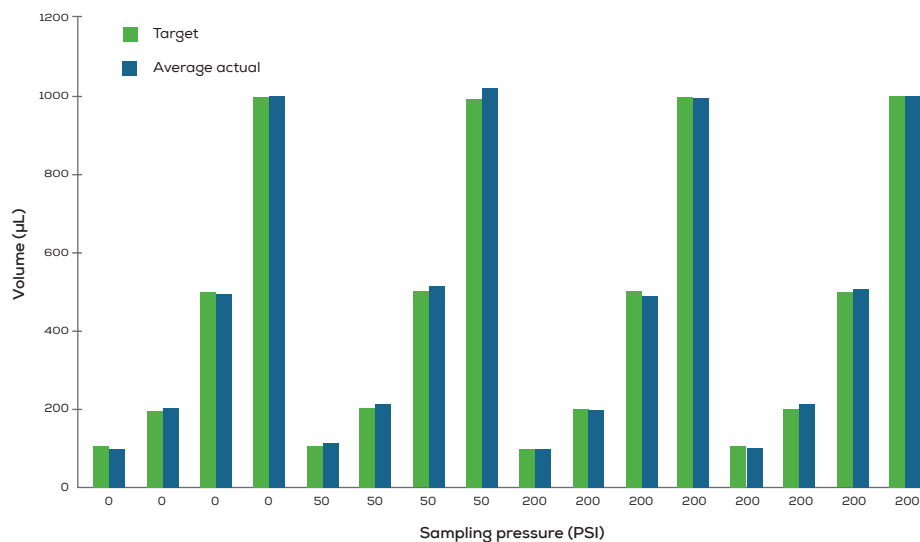
2-Methyltetrahydrofuran (2-MeTHF) as the solvent. The chemistry is not sensitive and both the starting material and the catalyst can be handled and weighed in a chemical hood. The conversion to Hydrocinnamic acid is always clean without detection of any impurity.

Sample variation testing

Prior to running the hydrogenation study, the precision and accuracy of pressure sampling was tested from 100–1000 µL under 0–400 psi of pressure using 2-MeTHF as the testing solvent (same solvent as for the hydrogenation reaction). Sample variation was less than 4% using the OSR (Figure 5).

5% Pd/C

5% Pd/C was first to be evaluated as the catalyst. The chemistry typically involves 5 mg of 5% Pd/C in 2-MeTHF at 40 °C under 50 psi of H₂.



Target volume (µL)	% RSD (precision)				% accuracy (average actual/target)			
	0 psi	50 psi	200 psi	400 psi	0 psi	50 psi	200 psi	400 psi
100	2.6%	0.3%	2.6%	3.1%	95%	105%	96%	103%
200	0.9%	1.1%	1.6%	1.1%	99%	102%	98%	105%
500	0.5%	0.3%	0.7%	0.9%	99%	101%	98%	102%
1000	0.2%	0.1%	0.6%	0.1%	100%	102%	100%	100%

Figure 5: Sampling precision and accuracy at pressure.

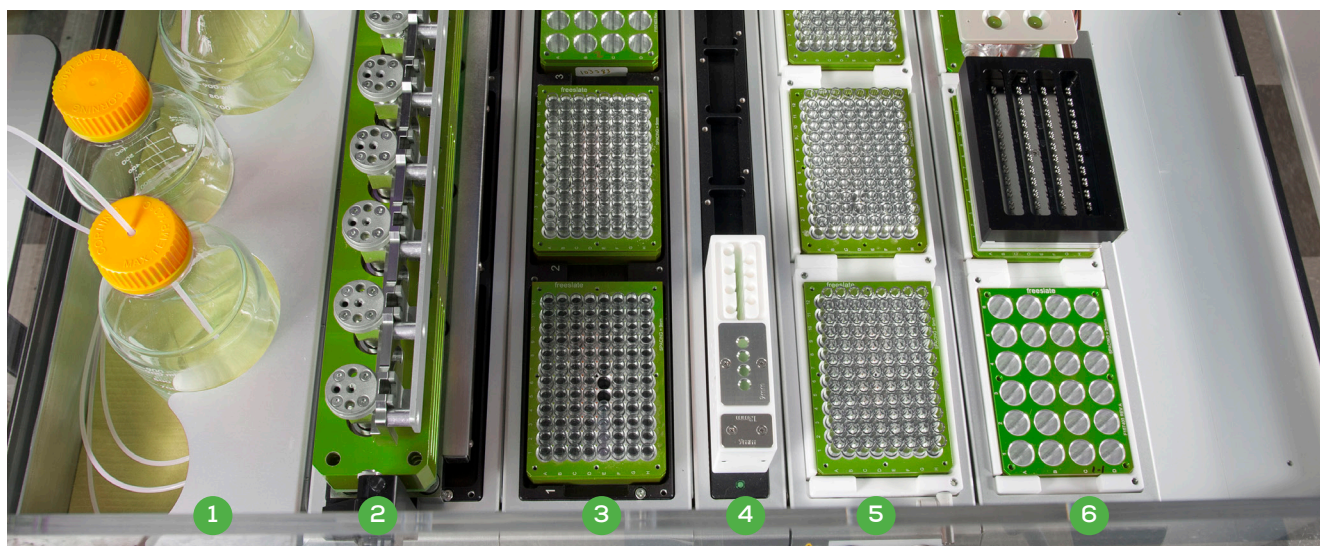


Figure 6: The freeslate jr. deck layout. (1) solvent tray, (2) OSR, (3) 3-position passive rack, (4) wash station, (5) heating/cooling/stirring station and (6) heating/stirring station.

Run Design:

- Start material: 20 mL 50 mg/mL *trans*-Cinnamic acid in 2-MeTHF solution
- Catalyst: 5 mg 5% Pd/C
- Process gas: H₂ at 50 psi
- Paddle speed: 500 rpm
- Sampling intervals (min): 0, 24, 48, 72, 96
- Analytics: HPLC after 50x dilution of samples with 30/70 THF/MeCN

Procedure:

- 1 Pre-weigh 5 mg 5% Pd/C and add to each reactor vial
- 2 Add 20 mL 50 mg/mL *trans*-Cinnamic acid solution to each reactor vial
- 3 Seal and purge the OSR with N₂
- 4 Start stirring at 500 rpm
- 5 Heat OSR to 40 °C, followed by 3 x 50 psi of H₂ purge to replace N₂
- 6 Take time series samples (100 µL) at stated intervals
- 7 Analyze HPLC after 50x dilution of samples with 30/70 THF/MeCN

5 mg of Pd catalyst was first added as powders to each OSR vial. It was followed by transferring 20 mL 50 mg/mL solution of *trans*-Cinnamic acid in 2-MeTHF to each vial. The OSR was sealed, and the heterogeneous solution stirred for approximately 10 minutes at room temperature while purging with nitrogen. A t=0, 100 µL sample was taken from each OSR reactor.

Once t=0 sampling was complete, the reactors were heated to 40 °C and purged with 3 x 50 psi of H₂ to replace existing N₂. The reactors were pressurized one more time with 50 psi of H₂ to commence the reactions. Samples (100 µL) were then taken from each reactor at 24, 48, 72 and 96 minutes. Once taken, samples were transferred to a microtiter plate located on the freeslate jr. deck (Figure 6) to be analyzed later by HPLC.

Results

The chemistry was done in about an hour. The percent conversion over time was then calculated based on HPLC data from each time point sampled (Figure 7). Standard deviation is less than 5% for all time points sampled.

$$\% \text{ Conversion} = \frac{\text{Product area (Hydrocinnamic acid)}}{\text{Total area (trans-Cinnamic acid + Hydrocinnamic acid)}}$$

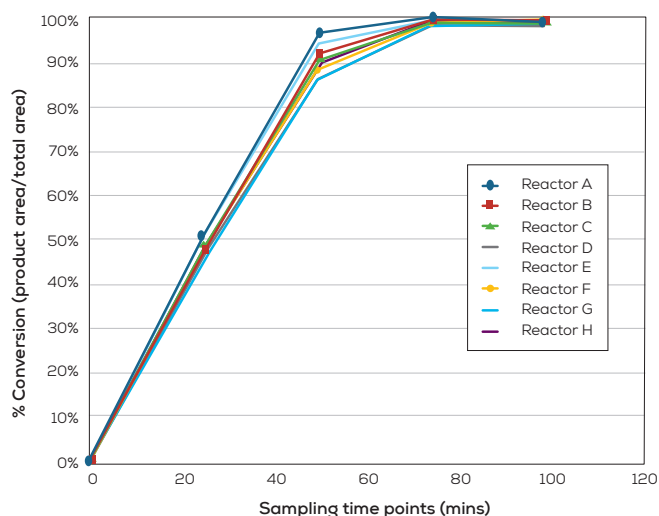


Figure 7: Percent conversion for 5% Pd Run based on HPLC data.

This Pd chemistry has been working well for us and we have been using it as part of our OSR development for a long time. There was just one noticeable issue. Depending on the locations, 5 mg 5% Pd/C can be difficult to accurately weigh due to static. Since overall catalyst amount is small, any inaccurate weighing would directly impact consistency in activity between reactors. As stated earlier, the ultimate goal was to find a very robust procedure that would reliably evaluate OSR performance with minimal difficulties conducting it in-house and at a customer site.

5% Rh on alumina on two different supports

One idea was to use less active catalysts so more catalysts are needed and variability in weighing becomes less of a factor. 5% Rh on alumina was chosen due to its known low activity. Two different catalyst supports, the matrix support (Aldrich

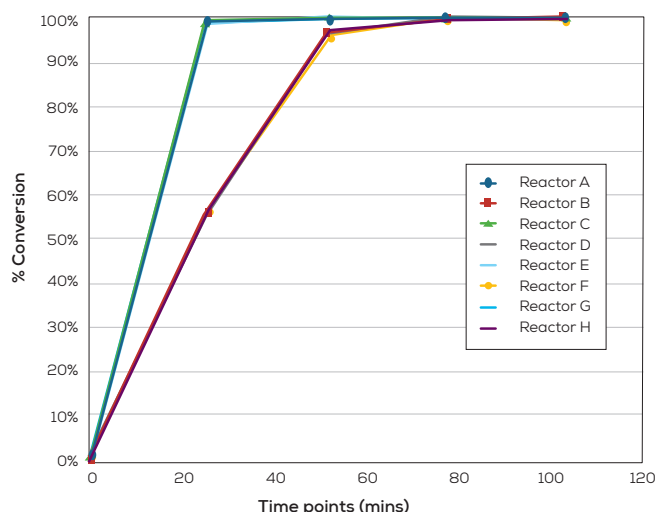


Figure 8: Percent conversion for 5% Rh on alumina run (two different supports) based on HPLC data.

212857) and the Degussa type support (Aldrich 663468) for 5% Rh on Alumina, were selected to gauge their effects on reaction rates. The study design includes four replicates for each support type (50 mg each) to assess consistency across different reactors (Table 1). The rest of the run design and procedure are the same as the 5% Pd run.

Reactions catalyzed by matrix-supported Rh (Reactor A, C, E and G) are clearly more active than Dugussa type (Reactor B, D, F and H) (Figure 8). All four replicates are also very consistent and the CV for all time points was <1% across eight reactors for all 48 time points.

Degussa Type 5% Rh on alumina

Slower Dugussa type catalyst is ideal for validation chemistry since it allows more evaluation (time) points before all reactions hit 100% conversion. The catalyst is also easy to weigh and has no static issues.

Reactor	A	B	C	D	E	F	G	H
Matrix support (mg)	50		50		50		50	
Degussa support (mg)		50		50		50		50

Table 1: Catalyst loading for 5% Rh on alumina run involving two different supports.

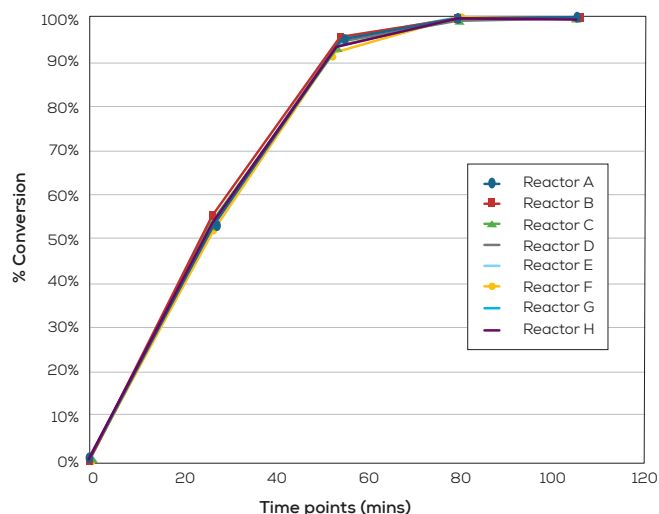


Figure 9: Percent conversion for 5% Rh on alumina run (Degussa support) based on HPLC data.

Validation chemistry based on Dugussa-support 5% Rh on alumina (50 mg) was then evaluated (Figure 9). Eight replicates were complete in about an hour (same as prior run) with great consistency (%RSD is less than 5% for all time point samples).

Using 50 mg Degussa type 5% Rh on alumina to catalyze hydrogenation of *trans*-Cinnamic acid reaction is currently our standard validation chemistry.

Conclusion

This study first demonstrated OSR's sampling capabilities at low volumes (100 μ L) and under high pressures (up to 400 psi) with sample variation at less than 4%. It was followed by three quick series of

reactions that successfully optimized a pressurized chemistry. For each series, a total of 40 (5 points x 8 reactors) time points were taken automatically *in-situ* and under pressure in two hours, where traditionally each individual time point must be set up as a reaction. The OSR's ability to gather significant amounts of data demonstrates how its use can contribute to a much greater understanding of reaction kinetics over a short period of time. In addition, the OSR's controlled sampling port technology allows experiments to be run in semi-batch mode where materials are added and taken away at pressure, which aligns with many plant-based operations where the addition of new reagents and sampling from the vessel can take place without halting the process.

Unchained Labs' reaction screening and catalysis optimization workflows for pharmaceutical process research, including OSR technology, allow scientists to increase the number of experiments they can perform while also improving the fidelity and traceability of their data. The workflows enabled by freeslate or freeslate jr. are compatible with a wide range of reactions and backed by Unchained Labs' many years of industry experience and domain knowledge. Scientists can comprehensively view and analyze all relevant data from initial design to analytics linked by a single experimental ID immediately or years from now. With these solutions, researchers can now get the right data and plenty of it in much less time, empowering development teams to make more informed decisions as processes are transferred and increase the chance for success at scale-up.



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